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Tailored Assays for the Detection of Agroterrorism Viral Agents

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National Nuclear Security Administration

Tailored Assays for the Detection of Agroterrorism Viral Agents

A collaborative effort between:

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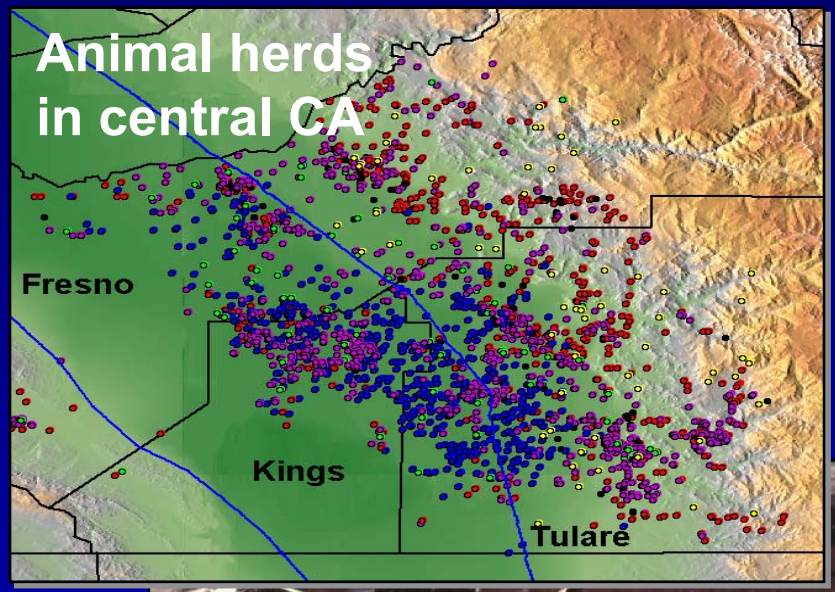
CDC Centers for Disease Control and Prevention

Agroterrorism

…a less publicized, but potentially devastating threat
(Henry Parker, NDU)

- ✓ **American food & agricultural infrastructure:**
  - **One-sixth of the national GDP**
  - **1 in 8 Americans employed in food production**
- ✓ **Agricultural infrastructure vulnerable**
  - **High density production**
  - **Easily accessible**
  - **Highly susceptible**

New efforts will offer better surveillance and response strategies: rapid rule-outs for FADs

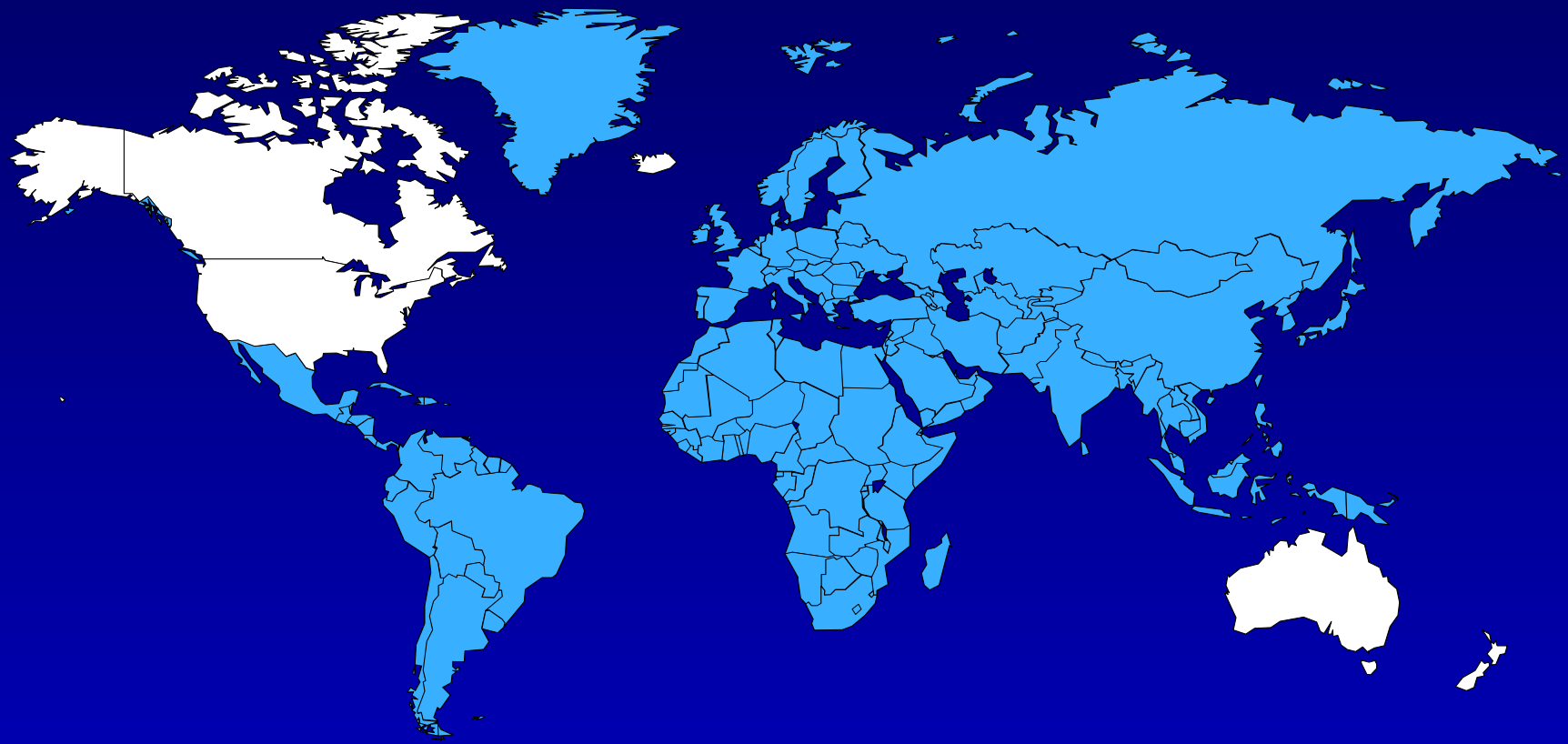


- ✓ Animals are highly vulnerable due to dense populations and lack of security
- ✓ Rapid diagnostics are needed to discriminate FADs from symptomatic look-alikes that naturally occur in our country
- ✓ Nested surveillance?

Motivation

- ✓ **Exotic agricultural diseases *will* be introduced**
  - **Naturally or intentionally**
  - **The question is not "if", but "when"**
- ✓ **Early detection is key to minimizing impact**
  - **Rapid, accurate detection technologies**
  - **Efficient, secure communication networks**

International Disease Status*



Office Internationale des Epizooties List A diseases: Foot and Mouth, Rinderpest, Classical Swine Fever, African Swine Fever, BSE, Exotic Newcastle disease, High Path Avian Influenza

A Coordinated Interagency Effort to Protect Agriculture

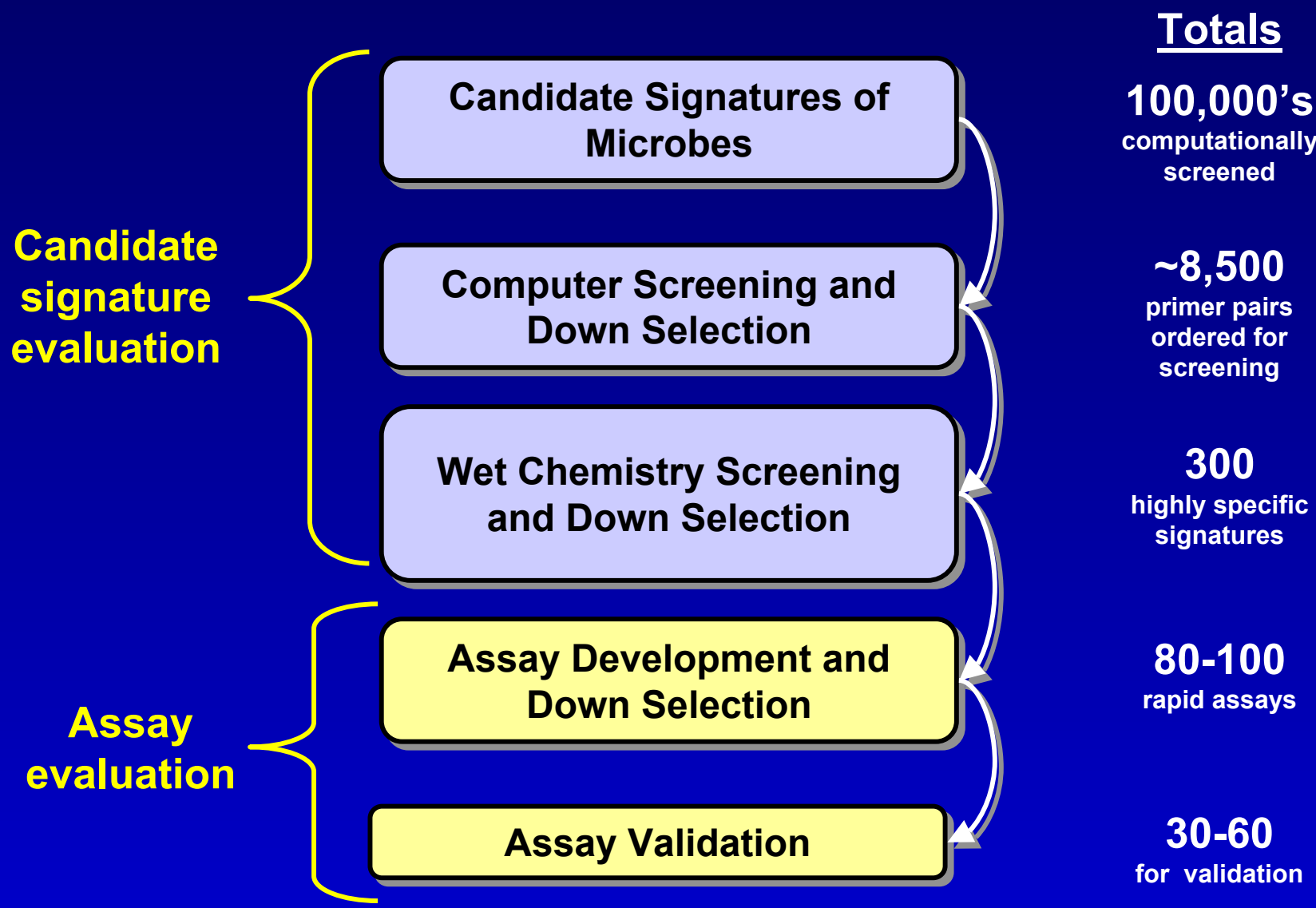
Rapid detection of exotic animal/plant diseases

- ✓ **Program objectives:**
  - **High degree of specificity**
  - **Compatible with several detection instruments**
  - **Validated for multiple plant/animal species**
  - **Disseminated through USDA/National Animal Health Laboratory Network**

Improved Diagnostics

- ✓ Work with collaborators to identify end user needs
  - Diagnostic gaps
  - Priority disease agents
- ✓ Leverage current infrastructure to deliver assays quickly
  - Tailored Assay program
  - LLNL whole genome approach
  - High throughput sequencing capability

The new process rapidly evaluates thousands of candidate signatures



Operational Strategy

- ✓ **Produce target list**
  - 8 viruses/ 2 bacteria
  - FMDV disease “look alikes”
- ✓ **Identify diagnostic platforms**
  - Specific & rapid
  - Cost effective & capable of high throughput
- ✓ **Develop signatures for bioassays**
  - Sequence target species & near neighbors
  - DNA pipeline for screening and down selection
  - End product is set of unique signatures

Symptoms of foreign animal diseases are often indistinguishable from endemic viruses

Foot and Mouth Disease

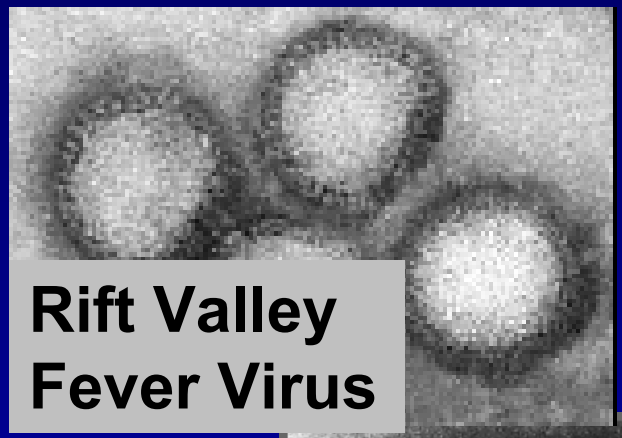
Vesicular Stomatitis

Malignant Catarrhal Fever

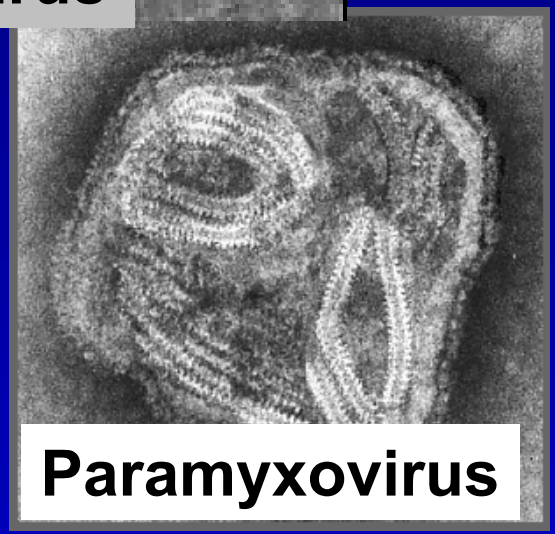
Bluetongue

BVD

This effort has expanded to support USDA on high priority assay development



Rift Valley Fever Virus

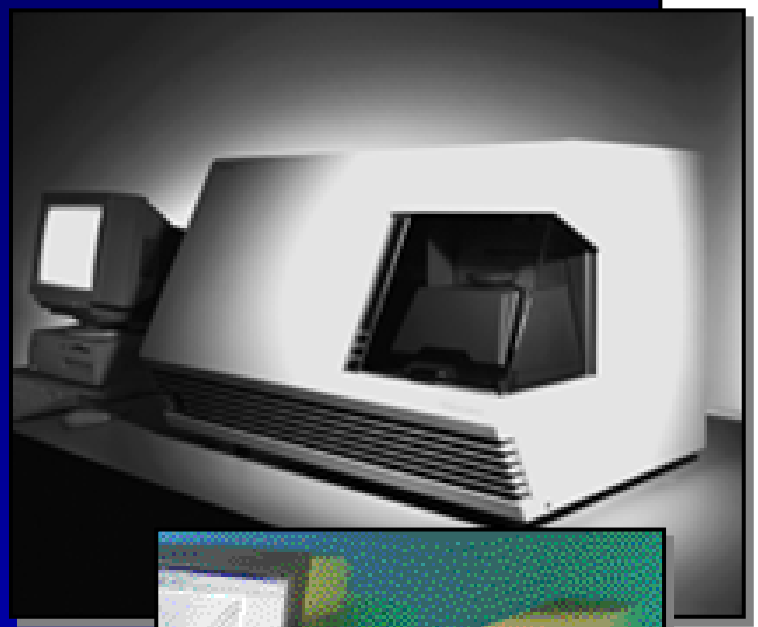


Paramyxovirus

- ✓ Listeria monocytogenes
- ✓ Orf
- ✓ Vesicular Exanthama of Swine
- ✓ Pseudorabies
- ✓ Johne's Disease
- ✓ Rift Valley Fever
- ✓ Infectious Bovine Rhinotracheitis
- ✓ Swine Vesicular Disease
- ✓ Malignant Catarrhal Fever
- ✓ Newcastle

This pathogen assay list was selected by USDA and reflects priorities and coordination with PIADC

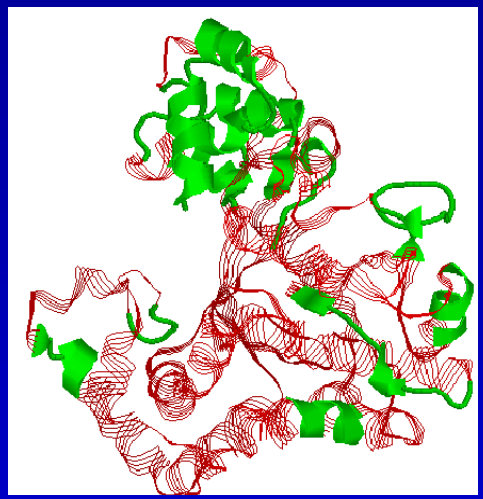
Signatures that pass screening are optimized for detection on different real-time PCR platforms



Sequencing efforts will produce the fundamental data upon which diagnostics are based

- ✓ Better characterization of pathogen diversity
- ✓ Improve throughput
- ✓ Increase cost effectiveness of whole genome sequencing of viruses

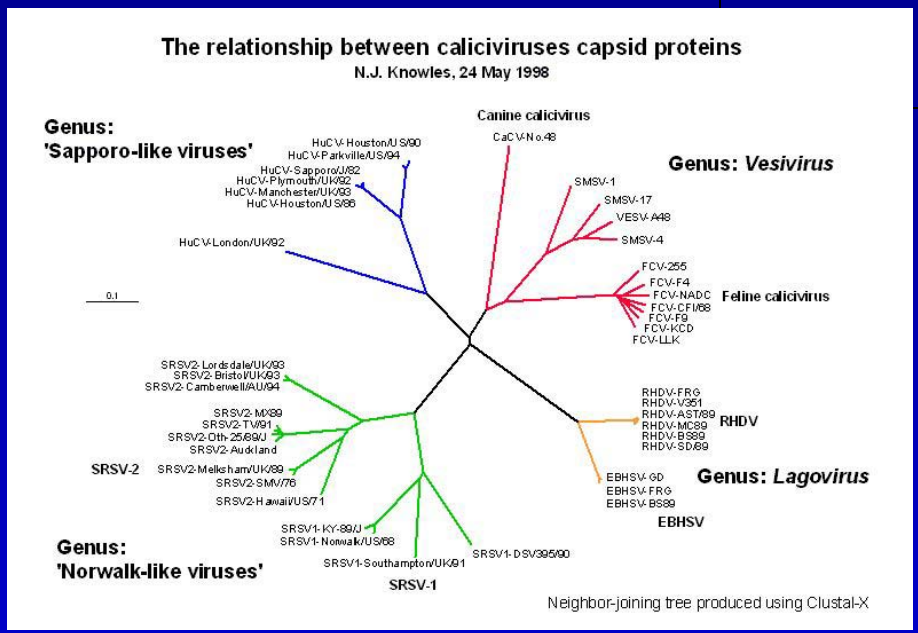
```
TCACTCCGGC CGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATA
TCACTCCGGC CGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATA
TTACTCCAGC TGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATT
TTACTCCAGC TGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATT
TCACTCCGGC CGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATA
TTACTCCAGC TGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATT
```



```
>fmdv.polymerase_P56:XXXNGTVGP
XVXXAXXLMXXXXYXXXXXTFLKDXX
RPXXXVRAGKTRXVXVXPVXXILYTXX
XXXVWXVDXSAFXXXXXXXAMNIMXX
XVXRTXXGXHPXAEWXLXTLVXTXHA
XXNXRXXVXGGMPSXXSATXIXNTILX
NXYVLYAXRXHXEGVELDTYTMISXXX
DXVVAXXXXXXXEALKPHXXSLGXTX
TPADKSDKGFVLGXSXTXVTFLKXHF
XXXXGTGFXKPVMASKTLEAILSFARR
GTXQEKLXSVAGLAVHSGPXXYRRLF
EPXXGLXEXPSYRSLYLRWVNAVCGD
X
```

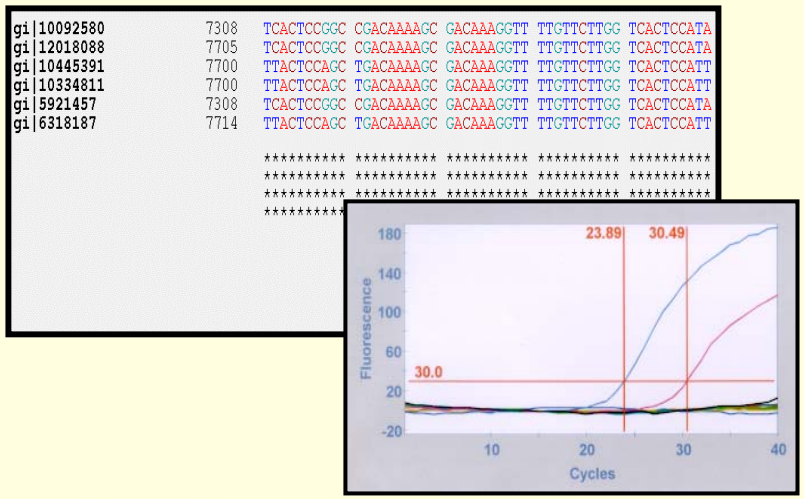
Justification for sequencing efforts…Improved bioassay signatures

- ✓ Greater accuracy
- ✓ Improved stability



✓ Rational approach to select near neighbors for sequencing

LLNL / CAHFS have been collaborating on FMDV / BVD assay development for almost 2 years



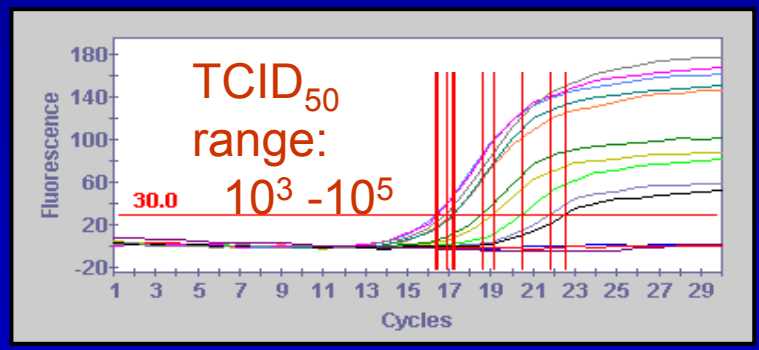
October 2001	November 2001	December 2001	January 2002	February 2002	March 2002	April 2002	May 2002	June 2002	July 2002	August 2002	September 2002	October 2002	November 2002	December 2002	January 2003

- **In Silico Development LLNL/CAHFS** (October 2001)
- **Initial Assay Testing FADDL-Plum Island** (February 2002)
- **Plum Island-Animal Experiments** (August 2002)
- **APHIS Validation Pending** (January 2003)

FMDV and BVD assays were developed in weeks

Which is FMDV?

- ✓ Rapid assays to rule out symptomatic “look-alike” diseases from FMDV
- ✓ Highly sensitive & specific
- ✓ Multiplexing has begun
- ✓ FMDV assays are in final validation at Plum Island



USDA making final decision on how to field assays of this type

LLNL / CAHFS provide in-depth diagnostic information within 2 days of receiving END suspect field samples

January 21, 2003 | January 22, 2003 | January 23, 2003 | January 24, 2003

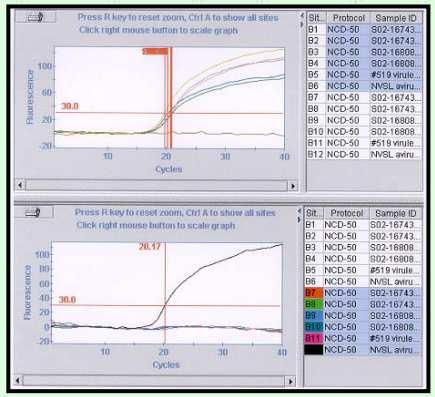
2 a m | noon | 12 a m | noon | 12 a m | noon | 12 a m | noon

10 am- Birds Submitted

Noon- Compatible Lesions

8 pm- Samples Arrive at Davis

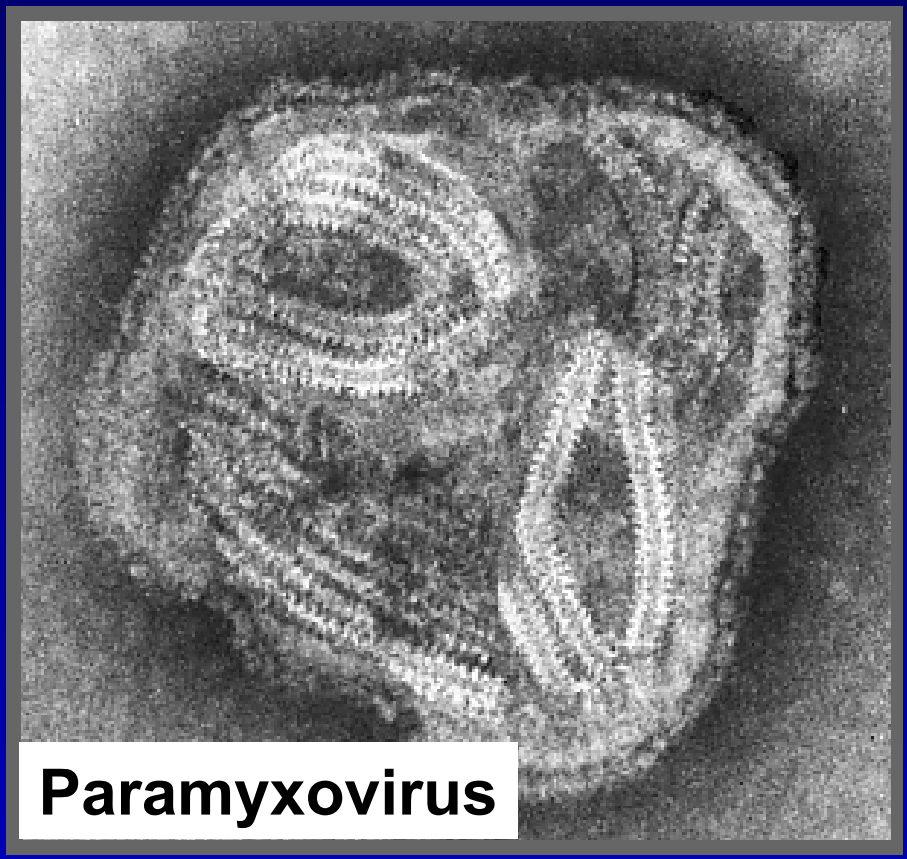
Midnight- +RT-PCR



11:45 am- Positive END Sequence

Noon- Virus Isolated +HA, HI

LLNL has demonstrated a capability to rapidly respond to emerging threats

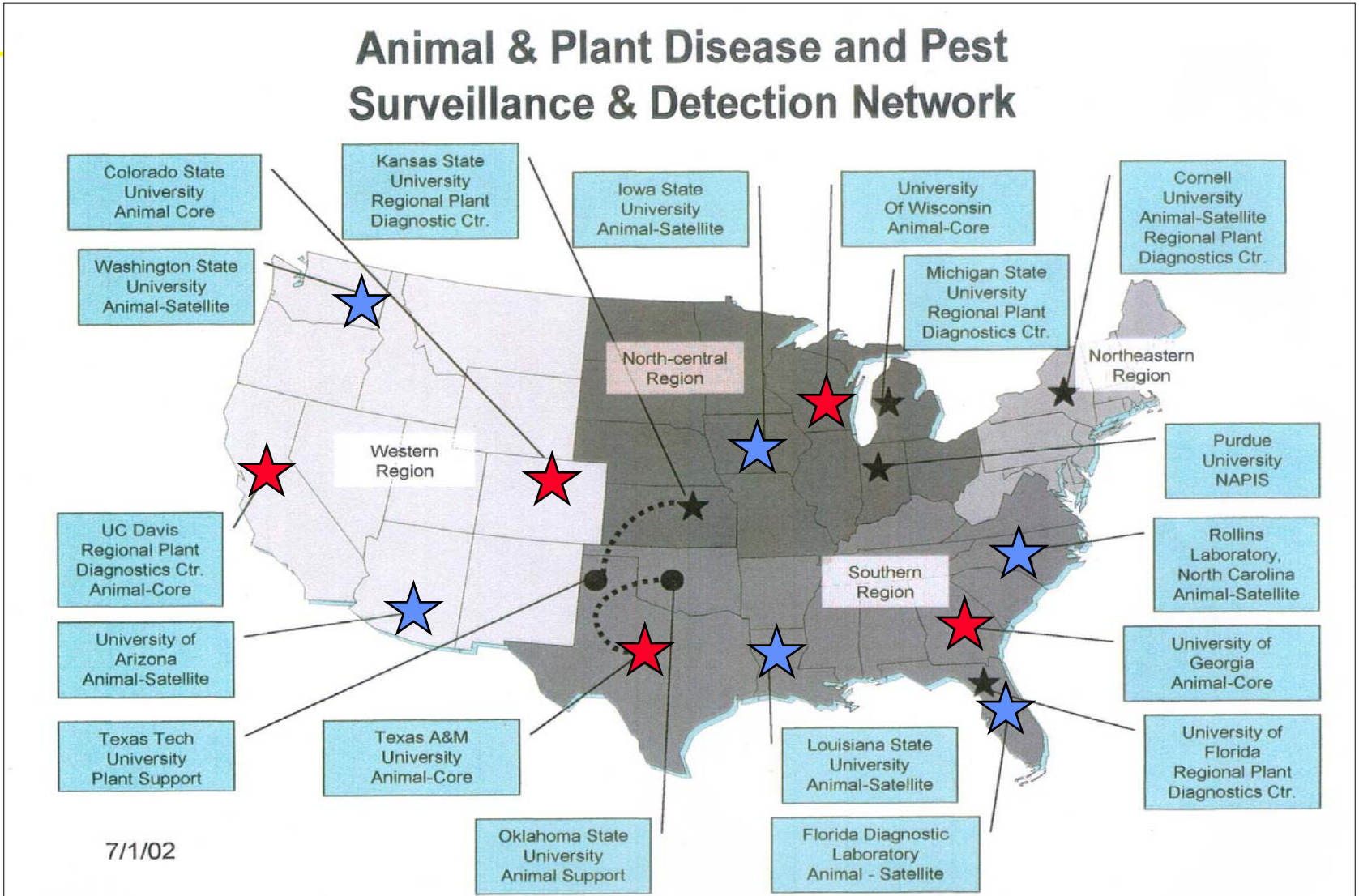


- ✓ Oct 13: UCD CAHFS Vet Lab called LLNL regarding Newcastle outbreak in game chickens
- ✓ Oct 15: Primers and probes ordered for screening
- ✓ Oct 18: Reagents split between vet diagnostic lab and LLNL screening lab
  - Vet lab will compare new assays with culture assays
  - LLNL will screen in parallel

Current testing takes about a week for feedback.

New process takes 1-2 hours.

USDA is networking animal and plant centers around the country



We are engaging the network for testing and validation

Future Directions

- ✓ FY'03 and beyond
  - Increase sequencing capabilities
    - Additional high priority pathogens
    - Denser sampling of near neighbors
  - Continued coordination with end users to field bioassays
    - validation
    - deployment
  - Investigate new technologies
    - High throughput
    - Field capable
    - Alternative DNA assays
    - Orthogonal testing - Protein signatures

Acknowledgments

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